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Genetic Analysis of Genealogy from Dental Y-STR DNA with Raman Spectra Method: Literature Review

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Abstract

The role of Forensic odontologist in genealogical analysis is important identification. This is useful in knowing whether someone has the same or different family lineage. Genealogical analysis can use Y-STR DNA from teeth with Amelogenin. This gene is present in hydroxyapatite crystals on the surface of enamel, dentin and tooth roots. Recent methods obtained from several literatures show that this examination can be performed using a Raman spectra technique which provides a laser wave stream during PCR analysis. The results of the conclusions from several literatures obtained good accuracy.

Key words: Forensic Odontologist, Genetic Genealogy, Y-STR, DNA, Y Chromosome, Dental Samples

Introduction

Forensic odontologist is a new and growing field of forensic medicine. Coupled with the application of technological advances to the detection and investigation of criminal activity, it facilitates the administration of the judiciary, which requires the coordinated efforts of interdisciplinary teams [1]. Personal identification using teeth is something that has been done for a long time [2]. It is hardest organ of human body and composed of pulp tissue contained in the enamel of the crown tooth and the hard layer of calcified dentin covered with root cement. These properties of the pulp provide high mechanical resistance to environmental invaders and surrounding microorganisms. The pulp is a non-calcified oral tissue composed of soft connective tissue, blood vessels, lymph, and nerve elements that occupy the pulp cavity of each tooth [3].

In connection with criminal cases, there is usually no evidence that can be used as a source of DNA testing in large quantities, but in limited numbers. So that the analysis of DNA examination samples really requires careful handling, considering that it will determine the success of subsequent DNA examinations. Bones and teeth are the strongest tissues of all human organs, which can be used as a

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source of forensic identification using DNA analysis[4]. Tooth debris (such as tooth samples) is generally affected by these variables and can be the only source of DNA if viable core DNA is not available for soft tissue degradation[3]. The odontologist may be asked to provide a sample for DNA analysis in most cases it can be a source of saliva, mucosal swabs, and teeth[5]. The implementation of an optimized workflow that combines extraction, quantification, and massively parallel sequencing (MPS) protocols was evaluated as access performance on forensic dental samples[3].

At the boundary between molecular genetics and genealogy is the genetic genealogy survey, also known as molecular genealogy. The high level of public interest in genealogy creating complex research disciplines may be due to the so-called identity crisis of globalization. Loss of identity, one of the main problems in the age of globalization, encourages people to find their place across generations. Information about your ancestors can help you find your lost ID. Molecular genealogy opens up the possibility of such identification with the help of DNA. This applied science can be thought of as a field of genealogy, known as social genetics, or historical genetics[6].

The genetic characteristics of the human Y chromosome provide markers of parent-child relationships in the form of a single haplotype that is passed directly from father to son. Haplotypes are a collection of short tandem repeat (STR) alleles typed on a single Y chromosome. For a long time, Y-chromosome analysis has been relatively neglected in forensic casework because it does not provide the security of identification that autosomal DNA can provide. The unilaterality of this marker can be useful in certain situations (sexual violence, missing persons, victim identification, complex kinship analysis, population reasoning) [7]. In the current study, the Raman spectral method is a molecule that can be used to identify gender identity by modeling PC scores, especially when modeling different tooth types (molars and premolars) separately. It is a test method[8]. Meanwhile, Kumari etc. (2017) Raman microspectroscopy is an alternative to age determination by using tooth samples of dentin and cement sections [9]. Based on a review of existing literature, the author is interested in reviewing several journals related to genetic genealogy analysis of tooth samples using YSTR-DNA and Raman spectral methods.

Raman Spectroscopy

Fourier Transform Infrared (FTIR) and Raman spectroscopy are vibration spectroscopy and serve as an ideal platform for identifying skeletal debris. Vibration spectroscopy can quickly and inexpensively extract large amounts of spectral information by capturing the vibrational motion of functional groups with simple sample pretreatment or without sample pretreatment [10].

Raman spectroscopy itself is based on Raman scattering or the Raman effect. That is, inelastic dissipation (about 1/106 to 1/108 photons) from visible monochromatic (400/700 nm) or laser (700/850 nm) or UV light (less than 270 nm). Due to the discrete changes in bright light above and below the wavelength of incident photons due to the vibrational frequencies (1/119) of the biomolecules that make up the tissue [11].

Raman spectroscopy has useful properties for forensic applications that identify body fluids (BFs) that non-invasively characterize a sample using light irradiation and highly selective spectral patterns based on the molecules that make up the sample. Detailed peak assignments show that the BF spectrum has a characteristic spectral pattern that can be interpreted with knowledge of the physiological components[12]. The use of Raman spectroscopy for biomedical applications, including dentistry, has increased significantly [11].

Raman spectroscopy and FTIR spectroscopy are sensitive to skeletal changes and provide viable techniques for estimating the end of postmortem changes. This makes this approach a diagnostic triage tool and could be a viable option for DNA analysis [10].

Y-STR DNA

Most human nuclear chromosomes are inherited from both parents; only the Y chromosome does not. The unique role of this chromosome as a genetically dominant sex-determining factor makes it a constitutive and male-specific haploid[13]. The human Y chromosome has two pseudoautosomal

regions (PARs) with a short Yp arm and a long Yq arm, separated by a centromere (Figure 1). PARs, the short arm and the proximal portion of the long arm consist of light chromatin enriched with the PAR1 and PAR2 genes, both of which recombine with areas on the X chromosome; the remaining region does not recombine and contains about 70 genes in the male-only region (MSY) [7].

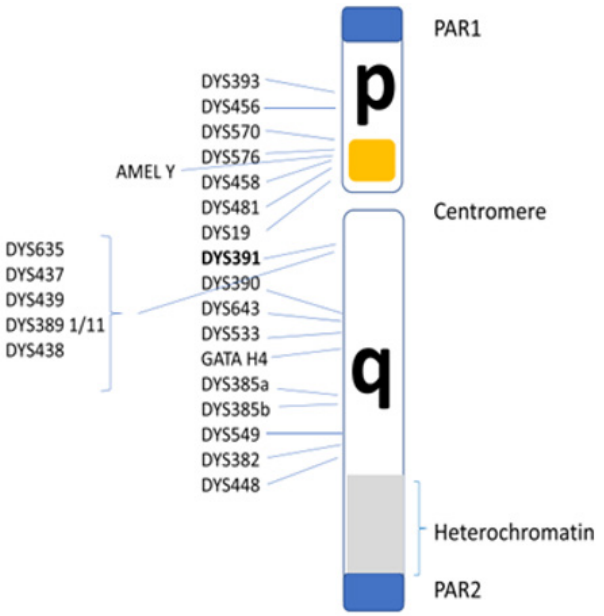

Figure 1: A structural diagram of the human Y chromosome identifying the relative location of the PowerPlex Y23 short-tandem repeat (STR) locus used routinely in forensic DNA casework. Estimated areas identified in diagrams are sometimes subject to variable size omissions.

Genetics Genealogy

Genealogical studies were conducted in the case of President Thomas Jefferson (1743-1826), a slave plantation in Virginia. This is an early application of the catalyst for the use of Y chromosome analysis in family history. The segregation of the Y-chromosome haplotype between the proven male ancestor of Jefferson's paternal uncle and his son's ancestor is suspicious[14].

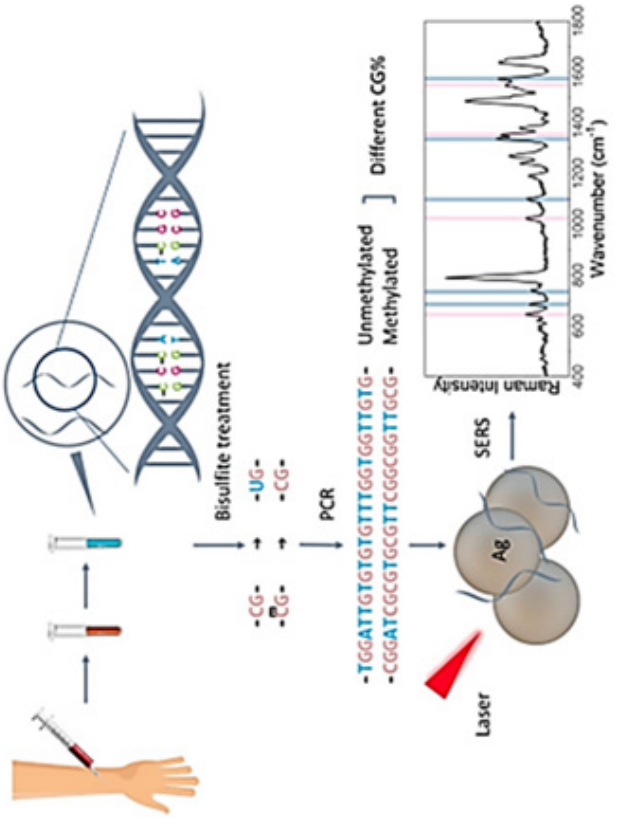

Figure 2: DNA Y analysis used the Raman spectro method

More generally, the relationships between the haplotype relationships of the Y chromosome names have practical implications. Predicting surnames based on the Y-chromosome haplotype is useful in unexpected criminal cases and has proven feasible in principle, but in practice it does have a related Y-chromosome haplotype. You need a very large database of family names you have [14]. Participant surnames appear to be predictable from published whole-genome sequence data when combined with published non-genetic data, thus raising privacy concerns regarding the anonymity of enrollment in medical genetics research [15].

MSY analysis is the gold standard for forensic researchers, and the most common approach is to use YSTR to determine if the required samples match. Learning family history is a very popular hobby, and DNA analysis has been enthusiastically accepted by the so-called pedigree genetics community [7]. The genetic characteristics of the human Y chromosome provide a parent-child relationship marker in the form of a single haplotype that is passed directly

from the father to the son. Haplotypes are a collection of short tandem repeat (STR) alleles typed on a single Y chromosome [16]. Y chromosome analysis has been relatively neglected for some time in forensic casework because it does not provide the close certainty of identification that autosomal DNA can offer. The uniparental nature of this marker can be useful in certain circumstances: sexual assault; missing person; identification of disaster victims; complex kinship analysis, and population inference [7].

Dental Amelogenin

Amelogenin accounts for about 90% of the total enamel matrix protein and plays an important role in enamel calcification and morphological changes [17]. This gene is found on the X chromosome of Xp22.1p22.3 and the Y chromosome of Yp11.2, with 90% of the transcript being expressed on the X chromosome and the rest on the Y chromosome. Homologous recombination has become the most popular genetic marker for sex determination in modern forensics [18].

Amelogenin Locus

This region encodes for extracellular matrix proteins involved in tooth enamel formation (Table 1). Mutations in the AMEL gene can cause an enamel defect known as amelogenesis imperfecta. Amelogenesis imperfecta is a disorder that causes abnormal tooth enamel formation in both primary and permanent teeth. The formed tooth enamel is soft and thin; so it is easily damaged [19].

Table 1: Sex Type Markers and Gene Homologs on Human X and Y Chromos

Sex-Typing Markers					
X Chromosome			Y Chromosome		
Gebe Symbol	Chromosomal Location	Distance from Xpter (Mb)	Gene Symbol	Chromosomal Location	Distance from Ypter (Mb)
AMELX	Xp22.2	11.3	AMELY	Yp11.2	6.7
DXYS156	Xq21.31	88.9	DXYS156	Yp11.2	3.2
SOX3	Xq27.1	139.6	SRY	Yp11.31	2.7
STS	Xp22.31	7.1-7.3	STSP1	Yq11.221	17.7
TSPYL2	Xp11.2	53.1	TSPY1	Yp11.2	9.2-9.3

The AMEL locus has two homologous genes: AMELX (Xp22.2) located on the human X chromosome and AMELY (Yp11.2) located on the human Y chromosome. Although the genes are homologous pairs, they differ in size and sequence [19].

The most commonly used gender typing method at the AMEL locus was the detection of a 6 bp deletion in AMELX intron 3. This deletion does not exist in AMELY. Sex can be determined using primers that specifically amplify the AMEL locus region. The primer set was developed to amplify both alleles in one PCR. The two most commonly used sets of amelogenin primers yielded 106 and 112 bp or 212 and 218 bp amplicons for the AMELX and AMELY loci, respectively. Amplicons generated from AMELX and AMELY were separated by electrophoresis. Observation of AMELX fragments only showed female, while observations of AMELX and AMELY fragments indicated male[19].

PCR

PCR was used amplify, the advent of polymerase chain reaction (PCR) has drastically changed the science of biology since it was first discovered. To amplify a small number of sufficient short target DNA sequences by utilizing sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase [20].

The DNA amplification techniques used were PCR, RTPCR, and nested PCR. Polymerase chain reaction (PCR) allows individual molecules of target DNA to be amplified to analytical amounts. A limited number of samples in the case of forensic medicine can be solved by changing the amplification. The number of PCR cycles was increased by 10 to properly amplify the degraded DNA [21]. This will result in 100% recovery of genomic DNA. RTPCR and nested PCR show high sensitivity, automation potential, and high throughput. RTPCR technology,

the X or Y chromosomes are identified by counting the number of chromosomes to determine sex. The X chromosome was counted using the comparative method 28 and the Y chromosome was counted using SRY [22].

Nested PCR studies have found that the sexual sensitivity of the amelogenin gene is 9.5-fold, based on the amelogenin ratio before and after nested PCR. Therefore, the degraded DNA can be analyzed by this method. The drawbacks of RTPCR, its susceptibility to false-positive results compared to PCR, and random dropouts when the number of copies is very small, can initiate real-time PCR and fail sex determination[23].

Amplification PCR Analysis of Amelogenin and Agerose Gel

Investigation of the amelogenin allele was performed using a different primer pair (for PCR amplification followed by agarose gel electrophoresis) than that provided by the AmpFISTR® IdentiferTM kit. The primer pair used amplifies different introns of one amelogenin gene. (X: p22.122.3 and Y: p11.2) Based on BLAST (Basic Local Alighmen Search Tool), a dot matrix showing similar regions of the AMELY gene and MALEX gene is displayed. A sequence of primers using capillary electrophoresis and primers using PCR amplification and agarose gel electrophoresis [29].

For PCR amplification with amelogenin primers, a disk with an FTA card section of approximately 1.2 mm (> 5 ng DNA) (after cleaning with whatman FTA cleaning reagent according to the manufacturer's protocol) or 1 ng DNA was used. Already used. The PCR reaction was performed in a volume of 25 ml. Each PCR reaction mix includes 1 ml serum bovine albumin (BSA) (2mg/ml), 12.5 ml TaqDNA Polyemerase Master MIX 2x (Qiagen), and 1 ml each amelogenin primer (forward and recognition 10 M) (Sigma). Make a final volume of 25 ml with 3 ml of template DNA (1 ng) or 2 FTA disks (> 5 ng DNA) and nuclease-free water [29].

The PCR reaction conditions were the same as those used to amplify the amelogenin gene using the AmpFISTR Identifier kit, and that amplicons were produced using modified primer pairs. PCR was performed on a thermosizer at an initial temperature of 95°C for 11 minutes, followed by denaturation at 95°C for 1 minute, annealing at 59°C for 1 minute, and elongation at 60°C for 1 minute for 27 cycles. Then hold at 60°C for 80 minutes at 4°C. PCR amplification bank (DNA replaced with an equal volume of extraction reagent blank control) was used as the negative control. The PCR product was analyzed at 120 V for 1 hour on a 1.5% agarose gel electrophoresis, then stained with ethidium bromide (Sigma) for 30 minutes and visualized under UV light[29].

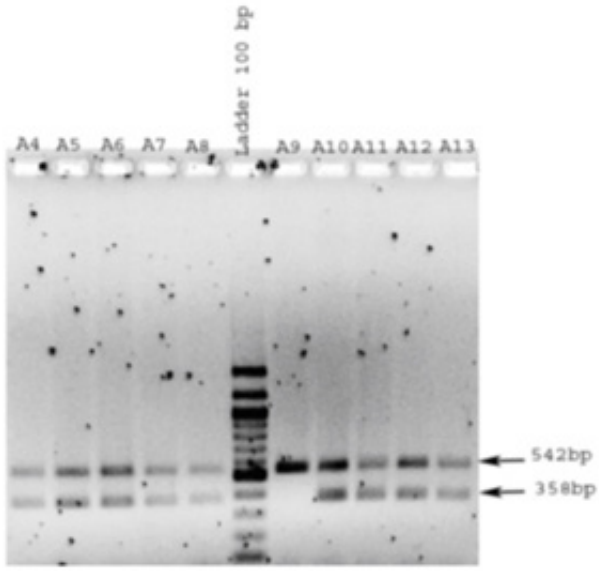

Figure 3: DNA of male samples using PCR primers amplification of the amelogenin gene and analysis by agarose gel electrophoresis.

Discussion

The human Y chromosome plays an important role in understanding human evolution and genetics. NRY is one of the most obvious regions of the human genome and is a powerful tool for studying paternal inheritance. NRY single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) have been used as important markers to track direct ancestors from paternal ancestors and reflect male historical behavioral traits [24]. In these cases, samples such as bone, teeth, skin, and muscle tissue usually provide sufficient DNA for analysis. In general, a 1 cm^2 section of such a biological material is suitable for testing [25].

Studies of Prikule et. al. (2021), Studying the Degree of Mineralization of Dental Enamel through

Raman Spectroscopy in Various Spectral Ranges, the method of vibration spectroscopy using IR Spectroscopy and Raman spectroscopy, the main method for studying various structures and properties of crystallized mineral compounds, such as tooth enamel and bone[26].

Gamulin et. al. (2021), conducted a study on gender analysis of dental samples using Raman spectroscopy on samples of premolars and molars. The results showed that the tooth root and neck anatomy could be used in sex analysis using a DNA molecular approach[8].

Meanwhile, Miyomari et. al. (2021), used Raman spectra in age analysis by imaging samples from the skin. Shows a less significant result, but is the latest result in a study using protein biomarkers in the skin[27].

Two of three studies conducted by Prikule (2021) and Gamulin (2021) mainly used examination of the amelogens of the tooth surface. In forensic DNA typing the detection of markers associated with amelogenin (AMEL). The locus has two homologous genes on the X and Y chromosomes: AMELY codes for a protein involved in demineralization of tooth enamel and has a paralogue on the X chromosome (AMELX). AMELX's first intron is six base pairs (bp) shorter and primers targeting both genes are included in many forensic DNA kits to reveal donor gender.

Sometimes the deletion will cover a wider area resulting in an incomplete Y chromosome STR locus in the profile routinely used in forensic analysis of the Y chromosome. This has led to other markers, such as DYS391, being included in standard autosomal DNA analysis kits because this marker resides in long arm of the Y chromosome and is not involved in the deletion event. Therefore, the examination of Y chromosomes and geneology (side) can be done using dental samples and also the spectrometric Raman method.

PCR examination was carried out with the addition of a laser as a tool for detecting spectra on the results of PCR analysis and to determine the significant similarity of results between gender Y in dental samples with DNA examination using the Raman spectra method in genealogy analysis.

Conclusion

Raman Spectra is a method that is quite old and can be used for the analysis of various biological components (blood, teeth, bones and skin) for DNA analysis. The combination of determining the family lineage of tooth samples using the Raman spectra method is still lacking in data in published research results. However, in the literature, the Raman spectra can be used in the detection of Y chromosomes from dental samples which can be used as a sideline identification.

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